REMARKS

Claims 1, 4-7, 10, 12-17, 19-21, 23-26, and 38-43 are pending in the application. Claim 4 is cancelled, its subject matter having been incorporated into Claim 1. Claim 43 is newly added.

Claims 1, 4-7, 10, 12-17, 19-21, 23-26, and 38-42 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Claims 1, 4-7, 10, 12-17, 19-20, 23-26, and 38-42 are rejected under 35 U.S.C. 103(a) as allegedly unpatentable over the article entitled "Optimization of Inclusion Body Solubilization and Renaturation of Recombinant Human Growth Hormone from *Escherichia coli*" in Protein Expression and Purification (18, 182-192, 2000) by Patra et el. ("Patra"), in view of the article entitled "Kinetics of inclusion body production in batch and high cell density fed-batch culture of *Escherichia coli* expressing ovine growth hormone" in J. Biotechnology (75, 161-172, 1999) by Panda et al. ("Panda"), and further in view of U.S. Patent No. 4,810,643 to Souza ("Souza"), U.S. Patent No. 5,618,927 to Ambrosius et al. ("Ambrosius"), U.S. Patent No. 5,773,581 to Camble et al. ("Camble"), and U.S. Publication No. 2002/0009798 to Pelleymounter et al. ("Pelleymounter"). Claim 21 was rejected under 35 U.S.C. §103(a) as allegedly being obvious from Patra in view of Panda, and further in view of Souza, Ambrosius, Camble, Pelleymounter, and U.S. Patent No. 6,677,139 to Donnelly et al. ("Donnelly").

Claim 1 is amended to specify the elected species. Claim 40 is amended to change the dependency from Claim 4 (now cancelled) to Claim 1. Claim 17 is amended to reflect the amendment made to Claim 1. Claims 12, 17, 19, 21, 23, 26 are further amended more particularly point out and distinctly define the claimed subject matter. New independent Claim 43 and dependent Claims 44-55 are added as an additional embodiment of the claimed subject matter. Support for the amendments may be found throughout the application and in the original claims, for example in original Claims 1-42. No new matter is added into the case by any of these amendments.

Each and every rejection of the claims and/or specification is respectfully traversed, and favorable reconsideration is requested in view of the foregoing amendments and the following remarks.

A. Claims 1, 4-7, 10, 12-17, 19-21, 23-26, and 38-39 are Not Indefinite.

Claims 1, 4-7, 10, 12-17, 19-21, 23-26, and 38-39 are said to be indefinite under § 112, second paragraph. Independent Claim 1 is directed to a process for the making a biologically

active protein, and Claims 4-10, 12-17, 19-21, 23-26, and 38-39 depend, directly or indirectly, from Claim 1.

It is alleged that Claim 1 is indefinite because it is said to be "unclear how the 'principle of performing the fermentation' can be regulated so as to perform the method step claimed." While not acknowledging that any of the present claims are indefinite for this or any other reason, in the interest of advancing prosecution of the case, Applicants offer an amendment to Claim 1 to address and overcome the alleged deficiency in the claims. As suggested by the Office Action, Claim 1 is now amended to recite "type of fermentation" instead of "principle of performing the fermentation."

It is believed the claims are now sufficiently definite under 35 U.S.C. §112, and that a person of ordinary skill in the art reading the claims would be reasonably well apprised of the metes and bounds of the claimed subject matter. Accordingly, amended Claim 1 is not indefinite, and reconsideration and allowance of the claim are respectfully requested.

Claims 3-7, 10, 12-17, 19-21, 23-26, and 38-39 are said to be indefinite for depending from an allegedly indefinite base claim. However, it has been shown above that the base claim, at least as amended, is sufficiently definite. Hence, the claims dependent thereupon should also be deemed compliant with §112 since their alleged §112 problems were said to stem only from the fact of their dependancy. Accordingly, reconsideration and allowance of Claims 3-7, 10, 12-17, 19-21, 23-26, and 38-39 are also respectfully requested.

B. Claims 1, 4-7, 10, 12-17, 19-20, 23-26, and 38-42 Are Patentable Over the Cited References.

Claims 1, 4-7, 10, 12-17, 19-20, 23-26, and 38-42 are said to be unpatentable over Patra in view of Panda, and further in view of Souza, Ambrosius, Camble, and Pelleymounter. Claim 1 is an independent claim, from which the remaining claims all depend.

Claim 1 is directed to a process for the production of biologically active G-CSF. Among other things, the G-CSF is expressed as a heterologous protein in a cultivated cellular expression system, while regulating the following cultivation parameters: temperature of cultivation, composition of cultivation medium, induction mode, type of fermentation, addition of a stress induction agent, and co-expression of auxiliary proteins. The protein is expressed as a substantially correctly folded protein precursor in non-classical inclusion bodies. The protein precursor also has an aqueous solubility. Regulating the cultivation parameters as called for

increases the proportion of substantially correctly folded protein precursor present in the non-classical inclusion bodies in the cell, relative to the proportion of substantially correctly folded protein precursor present in inclusion bodies in a cell of an organism not cultivated by regulating said parameters. Importantly, all of the recited cultivation parameters must be regulated conjunctively.

Claim 1 also calls for solubilizing the substantially correctly folded protein precursor from the non-classical inclusion bodies under non-denaturing conditions by contacting the non-classical inclusion bodies with a non-denaturing aqueous solvent having a pH of about 8.0. Additionally, the claimed process for the production of the biologically active G-CSF is free from any denaturation and renaturation of the G-CSF.

Patra is directed toward solubilization of r-hGH (recombinant Human Growth Hormone) from inclusion bodies using 100 mM Tris buffer and 2M urea at pH 12.5. Accordingly, the disclosure of Patra does not describe every limitation of the presently amended claims. Specifically, Patra fails to disclose the claimed limitations of "solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0," and that the process is free from any denaturation and renaturation of the G-CSF. Among other things, Patra teaches the use of higher pH conditions to achieve the desired solubilization.

Panda is directed toward a process for maximizing the volumetric productivity of recombinant ovine growth hormone (r-OGH) expressed in *E. coli*. Panda teaches solubilization of the protein from inclusion bodies using 50 mM Tris buffer (pH 10) containing 1% SDS. Accordingly, Panda fails to disclose, among other things, the claimed limitations of "solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0," and that the process is free from any denaturation and renaturation of the G-CSF.

In an attempt to remedy the deficiencies of Patra and Panda to provide all of the elements of the present claims, the Examiner combines the references with Souza, Ambrosius, Camble, and Pelleymounter. However, it is respectfully asserted that the combined references cannot be said to render Claim 1 obvious because a person of ordinary skill in the art neither could nor would combine the references in the manner suggested by the office action to arrive at the present

claims, for at least the reason that all the limitations of the present claims are not taught by the references.

Souza is directed to polypeptides sharing biological and immunological properties with human pluripotent G-CSF ("hpG-CSF"). Souza teaches that sequences coding for part or all of the sequence of amino acid residues of hpG-CSF or for analogs thereof may be incorporated into plasmid or viral vectors used to transform or transfect suitable prokaryotic or eukaryotic host cells such as bacteria, yeast or vertebrate cells in culture. Souza also teaches solubilization of the proteins using denaturing solutions (Souza, Example 7). Souza does not teach, disclose, or suggest the claimed limitations of "solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0," and that the process is free from any denaturation and renaturation of the G-CSF.

Ambrosius is directed to a process for the reactivation of a denatured protein (Ambrosius, Abstract). Ambrosius does not teach, disclose, or suggest the claimed limitations of "solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0," and that the process is free from any denaturation and renaturation of the G-CSF.

Camble is directed to pharmaceutical compositions of physiologically active polypeptides which provide continuous release of the polypeptide over an extended period when the composition is placed in an aqueous physiological-type environment. Camble teaches that it was found that the removal of detergent such as N-lauroyl sarcosine (in salt form) e.g. Sarkosyl activates a trace of proteolytic activity which may complicate product evaluation. It has further been found that this proteolytic activity may be significantly reduced and even eliminated if, after detergent removal by diafiltration, the pH is reduced to below 7.0 before substantial proteolysis, conveniently by diafiltration and preferably by dialysis. Thus the reduction or removal of trace proteolytic activity may be effected at a pH that is below 7.0 but which is sufficiently high to avoid significant hydrolysis of the polypeptide. The pH is advantageously in the range 6.0 to 4.5, preferably 5.8 to 5.0 especially about 5.4. Camble does not teach, disclose, or suggest the claimed limitations of "solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-

denaturing solvent having a pH of about 8.0," and that the process is free from any denaturation and renaturation of the G-CSF.

Pelleymounter is directed to methods and compositions for treating excess weight by administering OB protein. Pelleymounter teaches methods of refolding such proteins (Pelleymounter, [0031-0036]). Pelleymounter does not teach, disclose, or suggest the claimed limitations of "solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0," and that the process is free from any denaturation and renaturation of the G-CSF.

From the six combined references, a person of ordinary skill in the art would not be able to arrive at the present claims for at least the reason that none of the cited references discloses the claimed limitations of "solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0," and that the process is free from any denaturation and renaturation of the G-CSF. Since the references teach the use of denaturation/renaturation for the recover of proteins, a person of ordinary skill in the art following the combined teachings of the above references would much more likely arrive at a process involving denaturing and renaturing of the desired protein, rather than Applicants' claimed process. Furthermore, none of the cited references teach the specifically claimed combination of cultivation parameters that are regulated to in order to achieve production of the G-CSF in a high amount as in the present invention. Accordingly, claim 1 is patentable over the combination of Patra, Panda, Souza, Ambrosius, Camble, and Pelleymounter.

Since Claim 1 is shown to be non-obvious and patentable over the cited references, the claims dependent upon Claim 1 must also be patentable. Accordingly, reconsideration and allowance of Claims 1, 4-7, 10, 12-17, 19-20, 23-26, and 38-42 are hereby respectfully requested. In addition, new Claims 43-55 are patentable over the cited references for the same reasons that Claim 1 is patentable. Allowance of Claims 43-55 is hereby respectfully requested.

C. Claim 21 is Patentable Over the Cited References.

Claim 21 is said to be unpatentable over Patra in view of Panda, and further in view of Souza, Ambrosius, Camble, Pelleymounter, and Donnelly. However, Claim 21 is dependent

upon independent Claim 1, which was shown in part B above to be patentable over the combination of Patra, Panda, Souza, Ambrosius, Camble, and Pelleymounter. In order for a claim to be unpatentable over a combination of references, it must be shown, among other things, that the references contain at least all of the limitations of the rejected claim. It must also be shown that it would have been obvious to a person of ordinary skill in the art at the time the invention was made to put the references together in the manner set forth in the Office Action to arrive at the allegedly obvious claim.

Neither requirement is met here. Specifically, none of Patra, Panda, Souza, Ambrosius, Camble, Pelleymounter, or Donnelly teach or suggest the limitation (from Claim 1) of "solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0", and nothing in the combination teaches this sep carried out under these conditions. Even if the combination somehow contained this step, which it does not, nothing teaches combining these references in a way so as to provide a method containing this step.

Accordingly, since the combined references fail to render the independent claim unpatentable, dependent Claim 21 patentably distinguishes over the cited combination. Hence, reconsideration and allowance of Claim 21 are hereby respectfully requested.

CONCLUSION

Applicants respectfully submit that a full and complete response to the Office Action is provided herein, and that the application is now fully in condition for allowance. Action in accordance therewith is respectfully requested.

In the event this response is not timely filed, Applicants hereby petition for the appropriate extension of time and request that the fee for the extension along with any other fees which may be due with respect to this paper be charged to our Deposit Account No. 12-2355.

Respectfully submitted,
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